Acta Poloniae Pharmaceutica – Drug Research, Vol. 75 No. 2 pp. 525–531, 2018 ISSN 0001-6837
Polish Pharmaceutical Society

EVALUATION OF ANTIPLATELET ACTIVITY OF NOVEL GUANIDINE DERIVATIVES IN THE ASPECTS OF THEIR ADRENERGIC RECEPTOR ACTIVITY

AGNIESZKA ZAGÓRSKA[1]*, MONIKA MARCINKOWSKA[1], JOANNA ŚNIECIKOWSKA[1], ADAM BUCKI[1], AGATA SIWEK[2], MONIKA KUBACKA[3], GRZEGORZ KAZEK[4], JACEK SAPA[3], MAGDALENA KOTAŃSKA[3] and MARCIN KOŁACZKOWSKI[1]

[1]Department of Medicinal Chemistry, [2]Department of Pharmacobiology, [3]Department of Pharmacodynamics, [4]Department of Pharmacological Screening, Chair of Pharmacodynamics, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Kraków, Poland

Abstract: Designed acetamide derivatives based on guanidine and various heteroaryl carboxylic acids, were preliminary *in vitro* study of their adrenergic receptor affinity and anti-plateled effects. The obtained results have showed that exchange of 2,6-dichloro-phenyl substituent of guanidine into heteroaryl moieties, caused the decrease of receptor affinity, especially for α_1-adrenoceptors. The observed receptor profile of activity for α_{2B}-AR was not changed compared to α_1-ARs. Moreover, the observed effects on platelet aggregation induced by sub-threshold concentration of collagen and adrenaline strongly suggested that antiaggregant effect of N-(diaminomethylene)-2-(pyridin-3-yl)acetamide and N-(diaminomethylene)-2-(pyridin-4-yl)acetamide depends on their α_{2B}-ARs antagonistic activity.

Keywords: guanidine, α_1-adrenoceptors, α_{2B}-adrenoceptors, anti-aggregation, anti-platelet effects

The adrenergic system is a part of autonomic nervous system, which regulates neuronal, endocrine, metabolic and cardiovascular function. Adrenergic receptors (ARs), the members of G-protein coupled receptors (GPCRs) superfamily, are divided into three main classes α_1, α_2 and β (1). ARs of α_2 are a heterogeneous population, and at present three different subtypes α_{2A}-α_{2C} have been identified by molecular cloning in both the human and rat species. All three subtypes of α_2-ARs have been shown to inhibit the activation of adenylate cyclase and thus reduce the levels of cAMP (2, 3). The α_{2A} and α_{2C} subtypes are found mainly in the central nervous system (4). Stimulation of these receptor subtypes may be responsible for sedation, analgesia, and sympatholytic effects. The α_B-AR are found more frequently on vascular smooth muscle and have been shown to mediate vasopressor effects. All three α_2-AR subtypes represent potential cardiovascular drug targets and agonists of α_2-AR have been used for decades to treat common medical conditions such as hypertension; attention-deficit/hyperactivity disorder; various pain and panic disorders; symptoms of opioid, benzodiazepine, and alcohol withdrawal; and cigarette craving (5). Activation of α_{2C}-AR might have beneficial sympatho-inhibitory effects in hypertension and heart failure without the sedative side effects accompanying current clonidine-like drugs (6-8).

Compounds that are either agonists or antagonists of α_2-ARs have been discovered that represent a broad spectrum of chemical classes. Within these, a great range of selectivity has been demonstrated by physiologic and radioligand binding experiments. One of the most widely studied classes of α_2-AR ligands are the imidazolines (9, 10), which have been shown to be very peculiar in that small structural modifications can alter the balance between agonist and antagonist, as well as the α_1/α_2 selectivity. Representative examples of the most important structural types of α_2-AR -selective agonists and antagonists are shown in Figure 1.

On the other hand, platelet activation is a key factor in arterial thrombosis and antiplatelet therapy remains crucial in the therapeutic strategy for patients with cardiovascular diseases and high risk for a future

* Corresponding author: e-mail: agnieszka.zagorska@uj.edu.pl

cardiovascular event. Although controlled plug formation is necessary for the prevention of excessive blood loss, there are many pathological conditions in which the formation of occlusive thrombi may occur leading to severe clinical complications, including myocardial infarction and ischemic stroke (11, 12). Activation of sympathetic nervous system is also involved in the pathogenesis of thrombosis (11). Moreover, there were evidences that adrenaline has direct effects on platelets that are not mediated by ADP, endoperoxides or thromboxane A_2 liberated from platelets (13). It induces primary platelet aggregation, potentiation of stimulus-induced aggregation and secretion and inhibition of adenylate cyclase through activation of α_2-AR (14). The experiments of Steen et al. (15) proved that adrenaline *per se* does not induce human platelet activation *in vitro*. The platelet-stimulating effect of adrenaline through α_2-AR requires simultaneous activation by a true stimulatory platelet agonist. However, recent study indicated that α_{2B}-ARs exists in platelets and adrenaline induced amplification of platelet aggregation is at least in part mediated by activation of these receptors (11).

The aims of presented study were synthesis of designed acetamide derivatives based on guanidine and various heteroaryl carboxylic acids, and preliminary *in vitro* study of their adrenergic receptor affinity and anti-plateled effects. The assumption was to find anti-aggregation agents and eliminate side-effects on the cardiovascular system. The design of the new compounds using molecular modeling tools was carried out to introduce into structure of α_{2A}-AR agonist substituents maintaining high affinity and selectivity for α_{2B}-AR. Moreover, the studies were focused on compounds that the least possible affected on α_1-AR and penetrated the blood-brain barrier.

EXPERIMENTAL

Chemistry

Structures of the investigated compounds (**3a**-**3e**) and their syntheses are presented in Scheme 1. Compounds **3a**-**3d** were obtained by path A (Scheme 1) within a reaction of condensation of corresponding ethyl aryl-acetate (**1a**-**d**) with guanidine hydrochloride in isopropanol. Corresponding ethyl aryl-acetate derivatives (**1a**-**d**) were obtained according to the standard Fischer esterification procedure. Compound **3e** was obtained by path B (Scheme 1), by direct preparation of primary amides from carboxylic acid and guanidine hydrochloride using imidazole under microwave irradiation (16). The structure elucidations of the newly synthesized compounds were carried out using different spectroscopic techniques including ^{1}H and ^{13}C NMR and LC/MS. Further confirmations of the compounds were carried out by elemental analysis (± 0.4%). The elemental analysis data and some physical properties of these compounds are reported in the experimental part.

All the chemicals used were commercial products employed without purification. Purity of the synthesized compounds was confirmed by TLC performed on Merck silica gel 60 F_{254} aluminum sheets (Merck, Darmstadt, Germany) with the following solvents: A: dichloromethane/methanol (90 : 10), B: diethyl ether/dichloromethane/n-hexane/methanol (40 : 38: 20 : 0.2), C: dichloromethane/methanol/ammonia solution 25% (80 : 20 : 0.1). Spots were detected by their absorption under UV light (λ = 254 nm). Column chromatography separations were carried out on column with Merck Kieselgel 60 using the solvents A-C. ^{1}H and ^{13}C NMR were recorded at 300 MHz with Varian Mercury-VX spectrometer, in $CDCl_3$ or DMSO-d_6 solutions, using TMS (δ = 0.00 ppm) as an internal standard. The *J* values are in Hertz (Hz), and splitting patterns are designated as follows: s (singlet) and m (multiplet). LC/MS analyses were performed on Waters Acquity TQD apparatus with eλ DAD detector. For mass spectrometry ESI+ (electrospray positive) ionization mode was used. UV spectra were taken in 200-700 nm range. For establishing the purity of compounds, UV chromatograms were used. All investigated final compounds had purity over 95%. The LC/MS system consisted of a Waters Acquity UPLC, coupled to

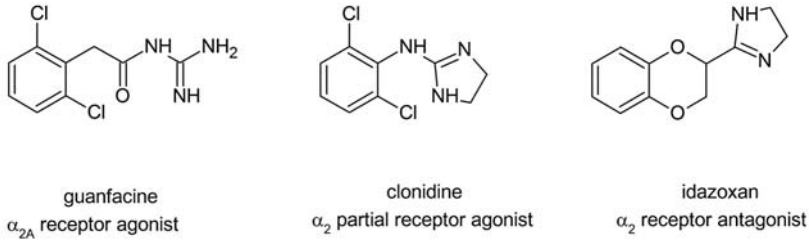
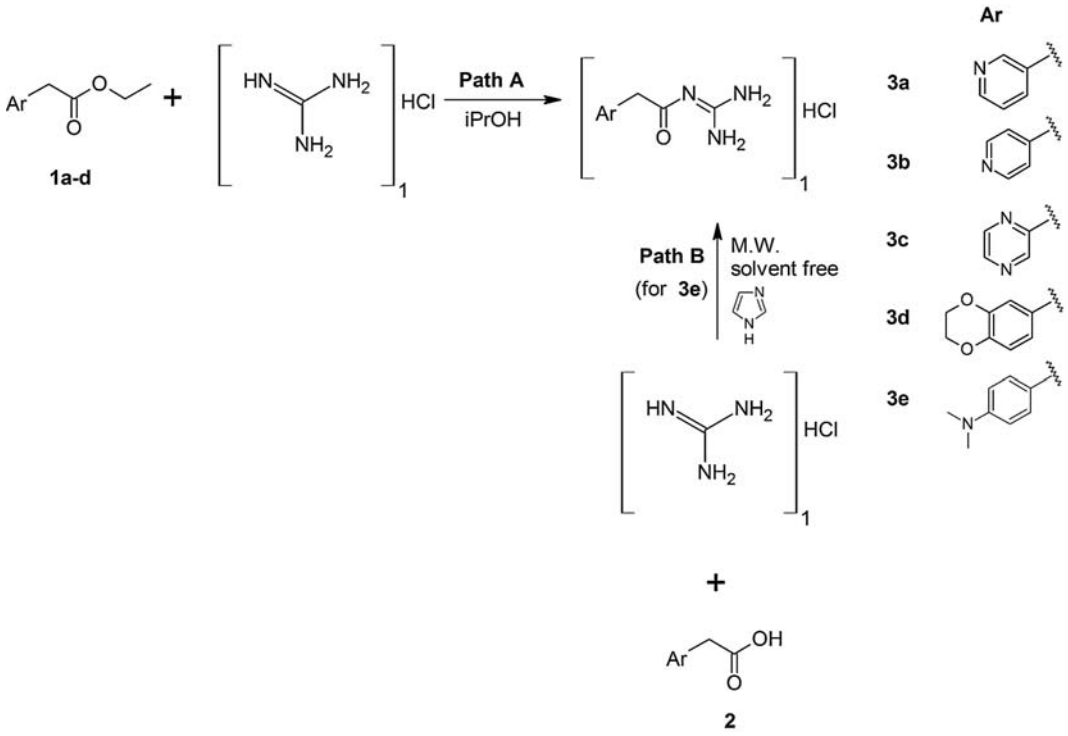


Figure 1. Chemical structures of potent α_2-AR ligands



Scheme 1. Synthetic pathways of novel acetamide hydrochlorides (**3a-3e**)

Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). All analyses were carried out using an Acquity UPLC BEH C18, 50 × 2.1 mm reversed-phase column. LC/MS data were obtained by scanning the first quadrupole in 0.5 s in a mass range from 100 to 700 m/z; 8 scans were summed up to produce the final spectrum. Elemental analyses were found within ±0.4% of the theoretical values. Reactions under microwave irradiation were carried out using a Discover LabMate (CEM Corporation). All products were isolated as water soluble hydrochloride salts (crystallizing oils).

General procedures for preparation of final compounds (3a-3d)

Mixtures of corresponding ethyl aryl-acetate (**1a-d**) (1 mM) with guanidine hydrochloride (1.1 mM) in isopropanol, were heated under reflux (60°C) for 72 h. After evaporation of the solvent to the brown oil residues, products were separated by column chromatography.

N-(Diaminomethylene)-2-(pyridin-3-yl)acetamide hydrochloride (3a)

From **1a** in 60% yield; Rf = 0.61 (A); ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.82-8.71 (m, 1H), 8.52-8.44 (m, 1H), 7.81-7.75 (m, 1H), 7.31-7.04 (s, 5H), 2.48 (s, 2H). ^{13}C NMR (DSMO-d_6, δ, ppm): 158.77, 153.98, 146.15, 141.35, 128.20, 124.83, 39.43; ESI-MS (*m/z*) 180.19 $(M+H)^+$. Analysis: calcd. for $C_8H_{11}ClN_4O$: C, 44.88; H, 5.17; N, 26.17%; found: C, 44.64; H, 5.36; N, 26.14%.

N-(Diaminomethylene)-2-(pyridin-4-yl)acetamide hydrochloride (3b)

From **1b** in 60% yield; Rf = 0.89 (B) ^{1}H NMR: ($CDCl_3$, δ, ppm): 8.51 (br. s., 4 H), 7.63 (dd, J = 1.8, 8.0 Hz, 2 H), 7.33 – 7.18 (m, 2 H), 3.63 – 3.56 (m, 2 H) ^{13}C NMR (DSMO-d_6, δ, ppm): 158.77, 153.98, 146.15, 141.35, 128.20, 124.83, 39.43; LC/MS *m/z* calcd.: 178.19 , found: 180.04 $(M+2H)^+$. Analysis: calcd. for $C_8H_{11}ClN_4O$: C, 44.76; H, 5.17; N, 26.10%; found C, 44.62; H, 5.38; N, 26.08%.

N-(Diaminomethylene)-2-(pyrazin-2-yl)acetamide hydrochloride (3c)

From **1c** in 51% yield; Rf = 0.90 (B);^{1}H-NMR ($CDCl_3$, δ, ppm): 8.79-8.72 (m, 5H), 8.59-8.51 (m, 2H), 3.94 (s, 2H), ^{13}C NMR (DSMO-d_6, δ, ppm): 169.36, 150.98, 144.61, 144.37, 142.06, 41.0; ESI-MS (*m/z*) 181.19 $(M+H)^+$. Analysis: calcd for $C_7H_{10}ClN_5O$: C, 40.37; H, 4.67%; N, 32.64%; found: C, 40.24; H, 4.63; N, 32.54%.

N-(Diaminomethylene)-2-(2,3-dihydrobenzo[b][1,4]dioxiny-6-yl)acetamide hydrochloride (3d)

From **1d** in 76% yield; Rf = 0.87 (C); ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.3 (s, 4H), 6.81-6.73 (m, 3H), 4.25 (s, 4H), 3.52 (s, 2H); ^{13}C NMR (DSMO-d_6, δ, ppm): 171.73, 143.38, 142.64, 127.26, 122.19, 118.03, 117.24, 64.30, 60.80, 40.63; ESI-MS (*m*/*z*) 237.16 $(M+H)^+$. Analysis: calcd for $C_{11}H_{14}ClN_3O_3$: C, 48.62; H, 5.19; N, 15.46%; found: C, 48.56; H, 5.30; N, 15.54%.

N-(Diaminomethylene)-2-(4-(dimethylamino)phenyl)acetamide hydrochloride (3e)

The mixture of N-(diaminomethylene)-2-(4-(dimethylamino)phenyl carboxylic acid **2** (1 mM), guanidine hydrochloride (2 mM) and imidazole (1 mM) was exposed to microwave irradiation with solvent free condition (100°C), power of MW oven (200 W) for 1 h (in 15 min intervals). The resulting crude product (**3e**) extracted was purified by column chromatography using solvents A system.

Yield 21%; Rf = 0.87 (C);^{1}H-NMR ($CDCl_3$, δ, ppm): 8.83-8.71 (m, 5H), 8.67-8.52 (m, 3H), 3.94 (s, 2H), 3.24 (s, 6H), ^{13}C NMR (DSMO-d_6, δ, ppm): 169.36, 150.98, 144.61, 144.37, 142.06, 134.6, 132.1, 114.9, 110.3, 41.0; ESI-MS (*m*/*z*) 221.27 $(M+H)^+$. Analysis: calcd for $C_{11}H_{17}ClN_4O_0$: C, 51.45; H, 6.67; N, 21.82%; found: C, 51.24; H, 6.63; N, 21.74%.

Pharmacology

***In vitro* binding assay**

Determination of the affinity of the tested compounds at the adrenoceptors

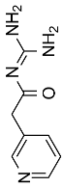
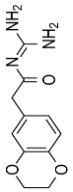
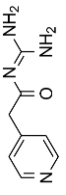
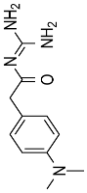
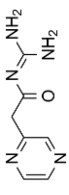
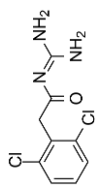
The affinity of the obtained compounds (**3a-3e**) was evaluated by radioligand binding assays (the ability to displace [^{3}H]-prazosin (spec. act. 85 C_i/mM, α_1 adrenergic receptor), [^{3}H]-clonidine (spec. act. 70.5 C_i/mM, Perkin Elmer, α_2 adrenergic receptor)) on rat cerebral cortex. The brains were homogenized in 20 volumes of an ice-cold 50 mM Tris-HCl buffer (pH 7.6) using an Ultra Turrax T25B (IKA) homogenizer and were centrifuged at 20 000 × g (15 048 rpm) for 20 min (0-4°C). The pellet was resuspended in the same buffer and centrifuged again. The final incubation mixture consisted of 240 µL tissue suspension, 30 µL of [^{3}H]-prazosin (0.2 nM) or [^{3}H]-clonidine (2.0 nM) solution and 30 µL of the buffer containing the investigated compounds. The concentrations of analyzed compounds ranged from 10^{-10} to 10^{-5} M. For measuring the unspecific binding, 10 µM phentolamine (in the case of [^{3}H]-prazosin) or 10 µM clonidine (in the case of [^{3}H]-clonidine) were applied. The following incubation parameters were applied: 30°C for 30 min (α_1 receptor) and 30°C for 25 min (α_2 receptor). The incubation was terminated by rapid filtration over glass fiber filters FilterMate B (PerkinElmer, USA) using 96-well FilterMate harvester (PerkinElmer, USA). Five rapid washes were performed with ice-cold 50 mM Tris-HCl buffer, pH 7.6. Filter mate was dried in microwave and placed in plastic bag (PerkinElmer, USA) and soaked in 10 mL of liquid scintillation cocktail Ultima Gold MV (PerkinElmer, USA). After even distribution of scintillation cocktail filter bag was sealed. The radioactivity on the filter was measured in MicroBetaTriLux 1450 scintillation counter (PerkinElmer, USA). All the assays were made in duplicate. Radioligand binding data were analyzed using iterative curve fitting routines GraphPad Prism 4.0 (GraphPad Software) using the built-in three parameter logistic model describing ligand competition binding to radioligand-labeled sites. The log IC_{50} (i.e., the log of the ligand concentration that reduces specific radioligand binding by 50%) estimated from the data is used to obtain the K_i by applying the Cheng-Prusoff approximation.

Functional assays for α_{2B}-adrenoceptors

Test and reference compounds were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1 mM. Serial dilutions were prepared in 96-well microplate in assay buffer and 8 to 10 concentrations were tested.

A cellular aequorin-based functional assay was performed with recombinant CHO-K1 cells expressing mitochondrially targeted aequorin, human GPCR for α_{2B} receptors. After thawing, cells were transferred to assay buffer (DMEM/HAM's F12 with 0.1% protease free BSA) and centrifuged. The cell pellet was resuspended in assay buffer and coelenterazine h was added at final concentrations of 5 µM. The cells suspension was incubated at 16°C, protected from light with constant agitation for 16 h and then diluted with assay buffer to the concentration of 100 000 cells/mL. After 1 h of incubation, 50 µL of the cells suspension was dispensed using automatic injectors built into the radiometric and luminescence plate counter MicroBeta2 LumiJET (PerkinElmer, USA) into white opaque 96-well microplates preloaded with test compounds. Immediate light emission generated following calcium mobilization was recorded for 30 s. In antagonist mode, after 30 min of incubation the reference agonist was added to the above assay mix and light emission was recorded again. Final concentration of

Table 1. The chemical structures of final compounds (**3a-3b**), guanfacine and their activity towards α_1- and α_{2B}-adrenergic receptors.

Compd	Chemical structure	[nM]*		Compd	Chemical structure	[nM]*	
		α_1	α_{2B}			α_1	α_{2B}
3a		> 1000	811.2 ± 134.6	**3d**		> 1000	1216 ± 658.7
3b		> 1000	320.0 ± 67.2	**3e**		> 1000	NA
3c		> 1000	1487 ± 603.4	**Guanfacine**		71.8	NA

*value of K_i for α_1AR, functional antagonist expressed as IC_{50} for α_2AR

the reference agonist was equal to EC80 (1.6 μM oxymetazoline).

Whole blood aggregation test

*In vitr*o aggregation tests were conducted using freshly collected whole blood with Multiplate platelet function analyzer (Roche Diagnostic), the five-channel aggregometer based on measurements of electric impedance. The Multiplate analyzer allows the duplicate measurement with dual electrode probes. Blood was drawn from carotid of rats with hirudin blood tube (Roche Diagnostic). 300 μL of hirudin anticoagulated blood was mixed with 300 μL prewarmed isotonic saline solution containing studied compound in DMSO or vehicle (DMSO 0.1% final) and preincubated for 3 min at 37°C with continuous stirring. The agonists (ADPtest, COLtest, Roche Diagnostic) were diluted using deionized water. Aggregation was induced by adding collagen (final concentration 1.6 μg/mL), or adrenaline and subthreshold concentration of collagen (final concentration 50 μM + 0.9 μg/mL). Activated platelet function was recorded for 6 min. The Multiplate software analyzed the area under the curve of the clotting process of each measurement and calculated the mean values.

Data were presented as the mean ± SEM. Statistical comparisons were made by the analysis of variance (ANOVA) and significance of the differences between control group and treated groups was determined by Dunnet *post hoc* test. $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The preparation of a novel group of molecules was carried out in a multistep reaction synthesis as shown in Scheme 1. The specific properties of the guanidine, causing inefficiency standard methods for amide bond formation. Guanidine is an exception to the general rule, when the fatty amines are less basic than amines saturated (pK_a = 13.6). Guanidine cation is formed in a polar solvent after the adoption of a proton from it, this form is more stable than the free base of guanidine. Therefore, optimization the synthetic method resulting various conditions (path A and B) for synthesis of compounds **3a-3e**. Compounds **3a**-**3d** were obtained by path A (Scheme 1) within a reaction of condensation of corresponding ethyl aryl-acetate (**1a**-**d**) with guanidine hydrochloride in isopropanol. Standard Fischer esterification (17) procedure was adopted for synthesis of ethyl aryl-acetate derivatives (**1a**-**d**). The corresponding carboxylic acid and an ethyl

alcohol were refluxed in the presence of an acid catalyst (hydrogen chloride gas). Compound **3e** was obtained by direct preparation of primary amides under microwave irradiation from carboxylic acid and guanidine hydrochloride.

The affinities of the newly prepared compounds were evaluated using radioligand binding assays (the ability to displace [^{3}H]-prazosin and [^{3}H]-clonidine from α_1-AR and α_2-AR, respectively) in rat cerebral cortex (18, 19). The majority of compounds tested not displaced [^{3}H]-prazosin and [^{3}H]-clonidine from cortical binding sites. The compounds were also tested in intrinsic activity studies using the human adrenergic α_{2B}-adrenoceptor expressed in CHO-K1 cells. Compounds **3a** and **3b** were found to be weak antagonist of α_{2B}-adrenoceptors (IC_{50} = 811 and 320 nM), whereas **3c** and **3d** have been found as very weak ligands (IC_{50} = 1487 and 1216 nM). Binding data are summarized in Table 1. The aims of this project was synthesis and evaluation of designed acetamide derivatives based on guanidine and various heteroaryl carboxylic acids. In order to achieve the desired property, 2,6-dichloro-phenyl substituent of guanidine was replaced with various heteroaryl moieties. Such modification, caused a significant decline in receptor affinity, especially for α_1 receptors. From the other hand, introduction of heteroaryl moieties did not change profile of functional activity towards

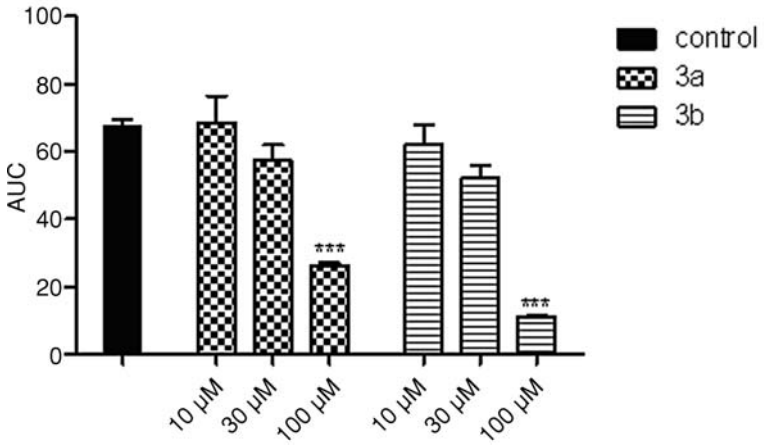


Figure 2. Effects of studied compounds on whole rat blood aggregation *in vitro* induced by collagen (1.6 µg/mL). Results are expressed as the mean ± S.E.M, n = 3, ***p < 0.001 *versus* control group (0.1% DMSO in saline)

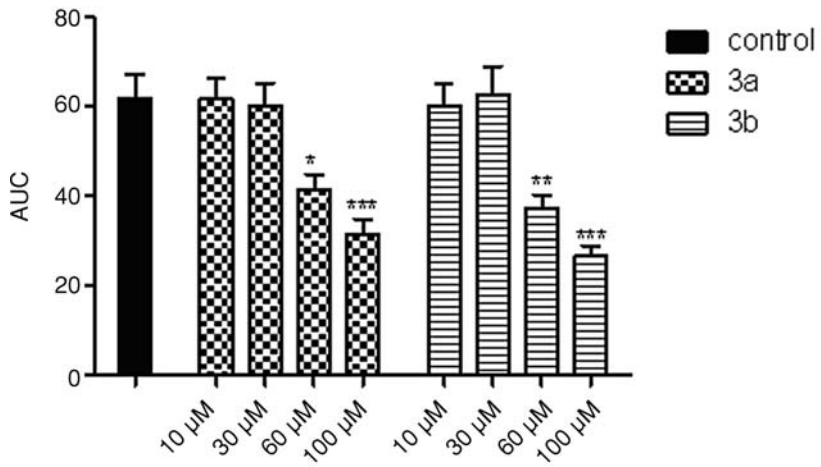


Figure 3. Effects of studied compounds on whole rat blood aggregation *in vitro* induced by simultaneous addition of adrenaline and collagen (30 µM + 0.9 µg/mL). Results are expressed as the mean ± S.E.M, n = 3, *p < 0.05, **p < 0.01, ***p < 0.001 *versus* control group (0.1% DMSO in saline)

α_{2B} adrenergic receptors, and compounds were active as receptor antagonists. Interestingly, the most active were compounds with pyridinyl moiety but connected with N-(diaminomethylene)acetyl fragment in different position of heterocyclic system (**3a** and **3b**). N-(Diaminomethylene)-2-(pyridin-4-yl)acetamide (**3b**) derivative was two more times active as antagonist, compared with pyridin-3-yl analog.

Next, the compounds **3a** and **3b** were evaluated for their antiplatelet effects in rat using *in vitro* method. Freshly isolated rat whole blood was incubated with tested substances (10-100 μM) or vehicle (DMSO). The aggregation responses were evaluated with Multiplate whole blood aggregometer by measuring impedance change. Platelet aggregation was induced by collagen or sub-threshold concentration of collagen and adrenaline. Compounds **3a** and **3b** were found to inhibit collagen induced platelet aggregation *in vitro* at the concentration of 100 μM, attenuating platelet aggregation by 83.3% and 61%, respectively (Fig. 2). Compounds were also tested in a model of adrenaline mediated amplification of collagen stimulated aggregation. Collagen at a concentration of 0.9 μg/mL did not aggregate rat blood *in vitro*, whereas adrenaline alone did not cause aggregation at any concentration tested. Combining adrenaline with the sub-maximal concentration of collagen produced a maximal aggregation response. Compounds **3a** and **3b** inhibited also aggregation induced by sub-threshold concentration of collagen and adrenaline (Fig. 3), reducing it at the concentration of 60 μM by 40.3% and 33.3%, respectively, and at the concentration of 100 μM by 57% and 49.5%, respectively.

CONCLUSION

The obtained results have showed that exchange of 2,6-dichloro-phenyl substituent of guanidine into heteroaryl moieties, caused the decrease of receptor affinity, especially for α_1-adrenoceptors. The observed receptor profile of activity for α_{2B}-AR was not changed compared to α_1-ARs. Moreover, the observed effects on platelet aggregation induced by sub-threshold concentration of collagen and adrenaline strongly suggested that antiaggregant effect of N-(diaminomethylene)-2-(pyridin-3-yl)acetamide and N-(diaminomethylene)-2-(pyridin-4-yl)acetamide depends on their α_{2B}-ARs antagonistic activity.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

Acknowledgments

These studies were financially supported by the National Science Center in Poland within the grant entitled "Partial agonists of alpha-2 adrenoceptors as a new perspective of the effective and safe in reducing body weight and obesity" (decision no. DEC-2011/03/B/NZ7/00635).

REFERENCES

1. Woodcock E.A., Du X.J., Reichelt M.E., Graham R.M.: Cardiovasc. Res. 77, 452 (2008).
2. Bylund B.D.: FASEB J. 6, 832 (1992).
3. Gentili F., Pigini M., Piergentili A., Giannella M.: Curr. Top. Med. Chem. 7, 163 (2007).
4. Giovannitti J.A.Jr., Sean M.T., Crawford J.J.: Anesth. Prog. 62, 31 (2015).
5. Gentili F., Cardinaletti C., Vesprini C., Ghelfi F. et al.: J. Med. Chem. 51, 4289 (2008).
6. Gilsbach R., Brede M., Beetz N., Moura E., Muthig V. et al.: Cardiovasc. Res. 75, 728 (2007).
7. Regitz-Zagrosek V., Hocher B., Bettmann M., Brede M., Hadamek K. et al.: Eur. Heart J. 27, 454 (2006).
8. Brede M., Wiesmann F., Jahns R., Hadamek K., Arnolt C. et al.: Circulation 106, 2491 (2002).
9. Ruffolo R.R., Waddell J.E.: J. Pharmacol. Exp. Ther. 224, 559 (1983).
10. Ruffolo R.R., Bondinell W.E., Hieble, J.P.: J. Med. Chem. 38, 3681 (1995).
11. Marketou M.E., Kintsurashvili E., Androulakis N.E., Kontaraki J., Alexandrakis M.G. et al.: J. Cardiol. 168, 2561 (2013).
12. De Meyer S.F., Vanhoorelbeke K., Broos K., Salles I.I., Deckmyn H.: Br. J. Haematol. 142, 515 (2008).
13. Siess W., Lapetina E.G.: Biochem. J. 263, 377 (1989).
14. Jackson S.P.: Blood 109, 5087 (2007).
15. Steen V.M., Holmsen H., Aarbakke G.: Thromb. Haemost. 70, 506 (1993).
16. Khalafi-Nezhad A., Mokhtari B., Soltani Rad M.N.: Tetrahedron Lett. 44, 7325 (2003).
17. Fisher E., Speier A.: Chemische Ber. 28, 3252 (1895).
18. Maj J., Klimek V., Nowak G.: Eur. J. Pharmacol. 119, 113 (1985).
19. Cheng Y., Prusoff W.H.: Biochem. Pharmacol. 22, 3099 (1973).

Received: 06. 02. 2017